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Kyla W. Taylor, Kate Hoffman, Kristina A. Thayer, and Julie L. Daniels

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Kyla W. Taylor,[1] Kate Hoffman,[2] Kristina A. Thayer,[1] and Julie L. Daniels[2]

[1]Office of Health Assessment and Translation, Division of the National Toxicology Program, National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services, Research Triangle Park, North Carolina, USA; [2]University of North Carolina Gillings School of Global Public Health, Chapel Hill, North Carolina, USA

Address correspondence to Kyla W. Taylor, National Toxicology Program, PO Box 12233, MD K2-04, Research Triangle Park, North Carolina USA 27709. Telephone 919-316-4707. Fax 919-541-1994. E-mail: taylorkw@niehs.nih.gov

Running title: PFCs and menopause among women from NHANES

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Abstract

Background: Polyfluoroalkyl chemicals (PFCs) such as perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) have been associated with early menopause. However, previous cross sectional studies have lacked adequate data to investigate possible reverse causality, i.e., higher serum concentrations due to decreased excretion after menopause.

Objectives: We investigate the association between PFOS, PFOA, perfluorononanoate (PFNA), and perfluorohexane sulfonate (PFHxS) and age at natural menopause among women ages 20-65 in NHANES.

Methods: We used proportional hazard models to estimate hazard ratios (HR) for the onset of natural menopause as a function of age and serum PFC levels, and to investigate reverse causation by estimating associations between PFC levels and rate of hysterectomy. We also used multivariable linear regression to determine whether time since menopause predicted serum PFC levels.

Results: After adjusting for age at survey, race/ethnicity, education, ever smoking, and parity, women with higher levels of PFCs had earlier menopause compared to women with the lowest levels. We observed a monotonic association with PFHxS: the HR was 1.42 (95% CI: 1.08, 1.87) for serum concentrations in the 2nd vs. 1st tertile, and 1.70 (95% CI: 1.36, 2.12) for the 3rd vs. 1st tertile. We also found evidence of reverse causation: PFCs were positively associated with rate of hysterectomy, and time since natural menopause was positively associated with serum PFCs.

Conclusions: Our findings suggest a positive association between PFCs and menopause; however, at least part of the association may be due to reverse causation. Regardless of underlying cause, women appear to have higher PFC concentrations after menopause.

Introduction

Polyfluoroalkyl chemicals (PFCs) are man-made compounds that have been used in a number of common consumer and industrial products such as food containers; stain- and water-resistant protection for clothing, furniture and carpets; paints; fire-fighting foam; and photographic emulsifiers (Lau et al. 2007). PFCs are ubiquitously present and persistent in the environment (Lau et al. 2007) and, although there are demographic, geographic, and temporal differences, exposures in the general population are widespread. Four PFC analytes: perfluorooctane sulfonate (PFOS), perfluorooctanoate (PFOA), perfluorononanoate (PFNA), and perfluorohexane sulfonate (PFHxS), are commonly detected in humans (Calafat et al. 2006; Fromme 2007; Kato 2011). Unlike traditional persistent organic pollutants (POPs) which are lipophilic and stored primarily in fat tissue, PFOS and PFOA are both lipophobic and hydrophobic. After absorption they persist in the body by forming chemical bonds to proteins in serum, rather than accumulating in lipids (Jones 2003; OECD 2002). Serum levels of PFCs reflect long-term exposures to these contaminants (EPA 2012b), with estimated geometric mean half-lives of 7.3 years (95% CI: 5.8, 9.2) for PFHxS, 4.8 years (95% CI: 4.0, 5.8) for PFOS, and 3.5 years (95% CI: 3.0, 4.1) for PFOA (GWBJMEDJFJ Olsen, W.; Seacat, Andrew M; Butenhoff, John L.; Zobel, Larry R 2007). However, a more recent study estimated a shorter median half-life for serum PFOA (2.3 years; 95% CI: 2.1, 2.4) (Bartell 2010).

PFCs are potential endocrine disrupters and effects of PFOS and PFOA on endocrine function have been reported based on animal studies (Jensen 2008; Zhao et al. 2010). Less is known about associations between PFCs and human endocrine function. Melzer et al. (2010) reported that higher concentrations of serum PFOA and PFOS were associated with current thyroid disease based on NHANES data from 1999-2000, 2003-2004, and 2005-2006. A positive association

between serum levels of PFNA and serum levels of thyroxine (T4) was reported in a population-based cohort of adolescents and young adults in Taiwan (Lin 2013). Yet, other studies have reported no association between PFOS or PFOA levels and thyroid function. These include occupational studies with high levels of exposure (Olsen 2003; GW Olsen 2007), a study of residents of a water district in Southeastern Ohio where there is significant environmental exposure to PFOA (Emmett 2006), studies of populations in Korea (Ji 2012), in China, (Lin 2013), among populations of Inuit adults (Dallaire et al. 2009), and among pregnant women (Chan et al. 2011; Inoue 2004). In the US, one of the largest efforts to investigate the impact of exposures to PFCs was initiated by the C8 Science Panel, which was created as part of a settlement agreement stemming from PFOA (or C8) contamination of drinking water in six water districts in two states near the DuPont Washington Works facility near Parkersburg, West Virginia (Frisbee et al. 2009). From this study, high environmental levels of PFOA in water were associated with delayed onset of puberty in girls (Lopez-Espinosa 2011) and earlier menopause. Knox et al (2011) found that serum PFOS and PFOA were significantly higher ($P<0.0001$) in women who had had a hysterectomy compared to other women 40-55 years of age. However, the timing of PFC exposure relative to menopause was not observed, thus causal inference is limited. One non-causal explanation for an association between PFCs and natural menopause is that elimination of PFCs via the loss of menstrual blood and tissue might result in lower serum levels in menstruating women than post-menopausal women.

We investigate associations between multiple PFCs (PFOS, PFOA, PFNA, and PFHxS) and age at natural and surgically induced (hysterectomy) menopause using National Health and Nutrition Examination Survey (NHANES) data. NHANES collected information regarding the age at

which women experienced menopause, which allowed us to investigate the relation between serum levels and time since menopause and the possibility of reverse causality.

Methods

Study Population

NHANES is a nationally representative, cross-sectional survey of about 5,000 persons each year. Survey participants are located in counties across the US. The survey uses in-home interviews and physical examinations in a mobile examination unit to collect data on demographics, behavioral and environmental risk factors, and health status (CDC 2012). Details regarding interview, examination, and sample collection protocols have been described previously. Written consent was obtained from participants after approval by the NCHS research ethics review board (CDC 2012). The University of North Carolina Institutional Review Board determined this study to be exempt from review because it is based on previously collected data that have been de-identified.

PFC Measurement

The National Center for Environmental Health analyzed individual serum PFC levels in five NHANES sample waves: 1999-2000, 2003-2004, 2005-2006, 2007-2008, and 2009-2010. In each sample wave, a random one-third subset of participants ≥ 12 years of age was selected for assessment of 12 PFCs (CDC 2009). Detailed analytic methods were described previously (Calafat 2007a, b; Kato 2011). Briefly, serum samples were analyzed using automated solid-phase extraction coupled to reverse-phase high-performance liquid chromatography/tandem mass spectrometry. The laboratory methods and comprehensive quality control system were consistent

across each NHANES wave, and documentation for each wave is available online (http://www.cdc.gov/nchs/nhanes/nhanes_questionnaires.htm). We examined PFOS, PFOA, PFNA, and PFHxS in relation to menopausal status because they were detected in >95% of samples, whereas other PFCs were detected infrequently (Calafat 2007a, b; Kato 2011). Any participant with a serum PFC concentration below the limit of detection (LOD) was assigned a serum level of the LOD divided by the square root of 2 (Calafat 2007a, b; Kato 2011). Serum PFC concentrations were categorized into tertiles based on distributions among all women in the study sample. We considered different categorizations, including quartiles and quintiles, but results were similar regardless of the categorization used (results not shown). We chose to categorize by tertile to increase the stability of our estimates.

Menopausal Status

Women age 18 and older completed a reproductive health questionnaire. They were asked: "Have you had at least one menstrual period in the past 12 months? (Please do not include bleedings caused by medical conditions, hormone therapy, or surgeries.)". Women who answered 'No' were subsequently asked: "What is the reason that you have not had a period in the past 12 months". We classified women as premenopausal if they answered 'Yes" to the first question or answered "No" to the first question but indicated the reason as due to pregnancy, breastfeeding, irregular periods, or medical conditions/treatments (N = 1800). We classified women as postmenopausal if they answered "No" to the first question and indicated the reason to be natural menopause (N = 501) or hysterectomy (N = 431). We excluded from all analyses 265 women with no information on their menopausal status and 14 who answered that they had not had their period in the last 12 months but did not state why (Figure 1). The distribution of demographic characteristics and PFC levels among these women were similar to the combined

larger sample except that they most were missing data for parity. Age at occurrence was recorded for women who reported having gone through menopause. We calculated time since menopause by subtracting the age at which post-menopausal women reported having their last menstrual period from their age at the time of survey.

Statistical Analyses

We assessed time to natural menopause using proportional hazard modeling of the hazard or rate of the onset of natural menopause as a function of age. Premenopausal women were censored at their age at the time of the survey. Analyses were performed in SAS (version 9.2; SAS Institute, Inc., Cary, NC) using the Proc SURVEYPHREG procedure, which accounts for stratification and clustering within primary sampling units used to select the NHANES sample. Rather than use NHANES sample weights, we adjusted all models for covariates related to the NHANES sample selection procedure (age and race/ethnicity), a method which balances the trade-off between efficiency and bias (Graubard and Korn 1999; Korn and Graubard 1991). To determine the robustness of our results to the inclusion of sample weights, we also conducted analyses using the NHANES sample weights. The inclusion of weights made no appreciable difference in the magnitude or precision of the associations estimated (results not shown), thus we present the un-weighted analyses.

Covariates

We considered a number of covariates as possible confounders in the association between PFCs and menopause. We used information in the literature on the association between other persistent organic pollutants and menopause to develop a directed acyclic graph (DAG) (Weng et al. 2009) that inferred the following confounders: Age, race, parity, education, and smoking (Figure 2)

(Cooper et al. 2002; Knox 2011). We did not adjust for BMI for several reasons. Steenland et al. (2009) reported an association between serum PFC levels and BMI which would make BMI a potential intermediate on the causal pathway between PFC exposure and menopause. In addition, high BMI has been associated with later onset of natural menopause (Akahoshi 2002). However, using data from NHANES, Nelson et al (2010) found no association between serum PFC levels and BMI, in which case, BMI would not meet criteria as a potential confounder (Greenland and Robins 1986). Additionally, BMI increases after menopause; thus, BMI reported at the time of the survey may not reflect BMI at the time of menopause. However, we tested to see if inclusion of BMI in our model appreciably altered the association between PFCs and menopause [hazard ratio (HR) change >10%] and found that it did not (results not shown). Adjustment for NHANES cycle was also considered. However, because cycle is strongly associated with serum PFC levels but not age at menopause it was not included as a covariate. Age at the time of interview was modeled as a continuous variable. Race/ethnicity, education, smoking, and parity were modeled as categorical variables (Table 1).

Reverse Causation

PFCs may be excreted in blood and tissue during menstruation. If so, women who are no longer menstruating may have higher levels of PFCs because they lack that elimination pathway. Consequently, any association between serum PFC levels and rates of natural menopause may be the result of reverse causation. That is, women who are no longer menstruating may have higher levels of PFCs because they lack the elimination pathway of menstruation. We investigated the potential for reverse causation in two ways. First, we used proportional hazard models to examine the association between PFC levels and rate of hysterectomy. Premenopausal women were censored at age of interview and postmenopausal women were censored at age of

menopause. If we assume that the reasons for hysterectomy are not related to PFC exposure, an observed positive association between PFC levels and hysterectomy suggest reverse causation. Conversely if there is no reverse causation, we would expect to see no association between levels of PFCs and hysterectomy. Second, we investigated whether rate of natural menopause predicts PFC levels. If the absence of menstrual blood and tissue loss explains the association between PFCs and self-reported natural menopause, we would expect women who menstruated more recently to have lower levels of PFCs in their serum. We used generalized additive models (GAMs) to examine the shape of the relationship between years since natural menopause and PFCs among post-menopausal women. Upon visual inspection, we determined that a linear approximation appropriately represented the shape of the relationship between time since menopause and PFC levels (results not shown). We report results using linear regression models (SAS version 9.2; SAS Institute, Inc., Cary, NC; Proc SURVEYREG). Statistical significance was defined as p-value = 0.05.

Results

Among the 2732 women with PFC measurements and menstrual status data, 65.9% (N = 1800) were premenopausal, 18.3% (N = 501) had completed natural menopause, and 15.7% (N = 431) had hysterectomies (Table 1). At the time of the survey, pre-menopausal women were generally younger (median age 34 years) and women who had gone through natural menopause were the oldest (median age 58 years). The median age at last period among women who completed natural menopause was 49 years. Similar to previous reports of the larger NHANES population, PFOS, PFOA, PFHxS, and PFNA were detected in at least 95% of serum samples from the women in our sample (Calafat 2007a, b; Kato 2009). The pre-menopausal group had the lowest

median levels of all PFCs, while the post-hysterectomy group had the highest median levels of PFCs (Table 1). Collection of serum and reproductive health questionnaires occurred years after natural menopause (median 7.5 years (interquartile range (IQR)) 8 years) and hysterectomy (median 14 years (IQR 16.5 years)). PFC measurements were also available for 265 women who lacked data on menstrual status and 14 who indicated they had not had a period in the past year, but did not report the reason.

Women with higher serum levels of PFCs consistently had higher rates of menopause compared to women with the lowest levels after adjusting for age at interview, race/ethnicity, education, smoking status, and parity (Figure 3). There appeared to be a monotonic dose-response association for menopause and PFOA, PFNA, and PFHxS. The adjusted hazard ratios for women with the highest levels (3rd vs. 1st tertiles) of PFOA, PFNA, and PFHxS in serum were 1.36 (95% CI: 1.05, 1.75), 1.47 (95% CI: 1.14, 1.90), and 1.70 (95% CI: 1.36, 2.12), respectively. The adjusted hazard ratios for the 2nd vs. 1st tertiles were 1.22 (95% CI: 0.92, 1.62) for PFOA in serum, 1.43 (95% CI: 1.07, 1.91) for PFNA in serum, and 1.42 (95% CI: 1.08, 1.87) for PFHxS in serum. For PFOS, adjusted hazard ratios were higher in the 2nd tertile than the 3rd tertile.

We found robust positive dose-response associations for all four PFCs and hysterectomy (Figure 4). PFHxS was most strongly associated with rate of hysterectomy (adjusted hazard ratio = 3.50 (2.72, 4.50)) in the highest tertile. Though the HRs are lower, the monotonic pattern of increasing HRs for PFOS and PFOA appears fairly similar to PFHxS. When using linear regression models to investigate whether time since natural menopause was associated with increasing PFC levels, we found that levels of all four PFCs increased with each additional year since natural menopause (Table 2).

Discussion

Higher body burdens of PFCs were associated with earlier onset of natural menopause. Associations were strongest between serum PFNA and PFHxS levels and rate of natural menopause. PFNA and PFHxS have not been studied previously with respect to menopause. This is of concern because PFNA and PFHxS have not declined over time in the same manner as PFOA and PFOS (Andersen et al. 2008; Calafat 2007b; Kato 2011); geometric mean serum levels of PFNA are increasing (from 0.55 to 1.49 ng/mL between survey years 1999-2000 and 2007-2008) and serum levels of PFHxS increased in the 2007-2008 cycle compared to previous years (Kato 2011). Our data also show positive associations between PFOS and PFOA and earlier menopause, consistent with previous reports in the literature (Knox 2011). As anticipated, serum levels of PFOA were lower in our sample (median = 3.8 ng/mL) compared to the C8 Health Study (median = 17.6 ng/mL in women 18 to ≤ 42 years and median = 23.4 ng/mL in women > 42 and ≤ 51 years) (Knox 2011), which had high levels of PFOA due to industrial contamination. Despite lower levels, we also observed a positive association between PFOA and the rate of natural menopause.

Although we observed associations for all assessed PFCs, we cannot rule out the possibility that associations are driven by a single congener, as some PFCs in sera are correlated Calafat (2007b) examined correlations using NHANES data and found statistically significant correlations ($p < 0.001$) between the log-transformed concentrations of PFOS and PFOA (Pearson correlation coefficient $r = 0.66$), PFHxs ($r = 0.56$), and PFNA ($r = 0.50$). The correlation coefficient between the log-transformed concentrations of PFOA and PFHxS was $r = 0.46$ and between PFOA and PFNA was $r = 0.55$.In the present data, Spearman correlations ranged from 0.19 between PFOS and PFNA to 0.65 between PFOS and PFOA ($P < 0.001$ for all correlations). We

considered the use of a total (summed) exposure measure of all four PFC congeners; however, PFOS levels were much higher in serum than PFOA, PFHxS, and PFNA, suggesting that the combined analysis would disproportionately reflect PFOS levels.

Early menopause is associated with a number of adverse health impacts. For example, results from a meta-analysis demonstrated that menopause before age 50 was associated with a 25% increased risk of cardiovascular disease (Atsma 2006) and menopause before age 46 has been associated with increased risk of coronary heart disease and stroke (Lisabeth et al. 2009; Wellons 2012). If PFC levels are predictors of earlier menopause, exposure may also increase the risk of other serious health outcomes (e.g. cardiovascular disease and stroke).

However, our investigation of reverse causality indicated positive associations between all four of the PFCs we examined and the rate of hysterectomy, and that these PFC levels increased with time since natural menopause. Taken together, the results of these two additional analyses suggest that the association between PFCs and menopause may reflect the accumulation of PFCs among women who were not excreting them through menstruation. However, due to the cross-sectional natural of our data we cannot confirm the direction of these associations. Prospective human studies evaluating the onset of menopause would be needed to better assess potential causality.

Using data from the large and US representative NHANES sample allowed us to explore the association between PFCs and the hazard of natural menopause while adjusting for potential confounding by a number of variables. Unlike previous analyses of PFCs and natural menopause, NHANES collected information on the time since natural menopause and surgical hysterectomy which allowed us to address the potential for reverse causality. The cross-sectional

nature of data collection does not allow us to establish temporality as menopause status, age of menopause, and PFC measurements were taken at the same time. PFC measurements were based on a single serum sample. Any misclassification from single measures would tend to decrease power and underestimate the real strengths of association (Pearce 2007). Although a single sample may more reliable for compounds with long half-lives, samples taken at several time points would be more accurate in classifying exposure in future studies (Melzer et al. 2010).

Conclusions

The consistency and robustness of our findings suggest that there is a relationship between PFCs and menopause, though the underlying mechanism of that association remains unknown. In these cross-sectional data, it is not clear whether the association observed between PFCs and menopause is causal, if results are due to non-causal influences such as biases due to confounding or misclassification, or if results are due to accumulation of PFCs post-menopause. Regardless of the underlying cause, women appear to accumulate PFCs more rapidly after they are no longer menstruating. These results, along with the ubiquitous nature of exposure and persistence of PFCs in the environment, support the need for continued monitoring of serum levels in the general population and further studies of the reproductive health effects of PFCs.

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Table 1. Descriptive statistics for women in NHANES 1999-2000, 2003-2004, 2005-2006, 2007-2008, and 2009-2010 describing women who currently have their period, women who have experienced menopause and women who have experienced hysterectomy.[a]

Characteristic	Pre-menopausal	Menopause	Hysterectomy
Total population (n)	1800	501	431
Age at interview (median ± SD)	34 ± 9.61	58 ± 5.25	55 ± 8.46
Age at last period (median ± SD)		49 ± 4.67	38 ± 7.66
NHANES Cycle [n (%)]			
1999-2000	282 (15.67)	67 (13.37)	48 (11.14)
2003-2004	298 (16.56)	84 (16.77)	98 (22.74)
2005-2006	403 (22.39)	83 (16.57)	75 (17.40)
2007-2008	389 (21.61)	125 (24.95)	110 (25.52)
2009-2010	428 (23.78)	142 (28.34)	100 (23.20)
Race/ethnicity [n (%)]			
White non-Hispanic	790 (43.89)	225 (44.91)	209 (48.49)
Black non-Hispanic	345 (19.17)	96 (19.16)	101 (23.43)
Mexican American	433 (24.06)	115 (22.95)	65 (15.08)
Other Hispanic	159 (8.83)	43 (8.58)	38 (8.82)
Other (including multi-racial)	73 (4.06)	22 (4.39)	18 (4.18)
Education [n (%)]			
< 12 years	440 (24.47)	158 (31.54)	112 (25.99)
12 years	359 (19.97)	100 (19.96)	127 (29.47)
> 12 years	999 (55.56)	243 (48.50)	192 (44.55)
Missing	2	0	0
BMI [n (%)]			
Underweight (< 18.5)	46 (2.56)	6 (1.21)	4 (0.94)
Normal weight (18.5 to < 25)	569 (31.74)	129 (26.01)	72 (16.98)
Overweight (25 to < 30)	516 (28.76)	140 (28.23)	123 (29.01)
Obese (>30)	663 (36.96)	221 (44.56)	225 (53.07)
Missing	6	5	7
Ever Smoked [n (%)]			
No	1188 (66.00)	270 (53.89)	230 (53.36)
Yes	612 (34.00)	231 (46.11)	201 (46.64)
Missing	0	0	0
Parity [n (%)]			
0 live births	388 (23.08)	62 (13.11)	39 (9.24)
1 live births	339 (20.17)	66 (14.00)	60 (14.22)
> 1 live births	954 (56.75)	344 (72.89)	323 (76.54)
Missing	119	29	9
PFC exposure [median (T1, T3)]			
PFOS (ng/mL)	10.3 (6.0, 17.0)	14.03 (8.80, 23.9)	17.50 (10.6, 29.4)
PFOA (ng/mL)	2.70 (1.80, 4.20)	3.80 (2.50, 5.30)	4.20 (2.90, 5.90)

Characteristic	Pre-menopausal	Menopause	Hysterectomy
PFNA (ng/mL)	0.90 (0.60, 1.40)	1.20 (0.80, 1.80)	1.30 (0.80, 2.10)
PFHxS (ng/mL)	1.00 (0.60, 1.80)	1.50 (0.90, 2.60)	1.70 (1.10, 3.10)

[a]Women with unknown menopausal status have been excluded. Abbreviations: T1- 1st tertile; T3- 3rd tertile.

Table 2. Adjusted β (95% CI)[a] for the change in serum PFC concentrations (ng/mL) associated with a 1-year increase in the time between natural menopause and sample collection among naturally post-menopausal women (n = 501), NHANES 1999-2000, 2003-2004, 2005-2006, 2007-2008, and 2009-2010.

PFC	**β (95% CI)[a]**
PFOS	0.23 (-0.16, 0.48)
PFOA	0.07 (0.013, 0.13)
PFNA	0.02 (0.002, 0.042)
PFHxS	0.023 (-0.019, 0.065)

[a]Adjusted for age at time of survey, race/ethnicity [White, African-American, Mexican American, other Hispanic, and Other (including multi-racial)], education (< 12 years, 12 years, and > 12 years), smoking (ever or never), and parity (0 live births, 1 live birth, > 1 live birth)

Figure Legends

Figure 1. Study population of women ages 20-65 with PFC serum measurements from NHANES years 1999-2000, 2003-2004, 2005-2006, 2007-2008, and 2009-2010.

Figure 2. Directed acyclic graph of association between PFC exposure and age of natural menopause.

Figure 3. Adjusted hazard ratios for menopause in association with tertiles of serum PFCs among women from NHANES 1999-2000, 2003-2004, 2005, 2006, 2007-2008, and 2009-2010. Based on proportional hazards model for age at menopause, censoring at interview age if still menstruating, and elimination all cases of hysterectomy. Hazard ratios adjusted for age at interview, race/ethnicity (White, African-American, Mexican American, other Hispanic, and Other - including multi-racial), education (< 12 years, 12 years, and > 12 years), smoking (ever or never), and parity (0 live births, 1 live birth, > 1 live birth).

Figure 4. Adjusted hazard ratios for hysterectomy in association with tertiles of serum PFCs among women from NHANES 1999-2000, 2003-2004, 2005, 2006, 2007-2008, and 2009-2010. Based on proportional hazards model for age at hysterectomy, censoring at interview age if still menstruating, and elimination all cases of hysterectomy. Hazard ratios adjusted for age at interview, race/ethnicity (White, African-American, Mexican American, other Hispanic, and Other - including multi-racial), education (< 12 years, 12 years, and > 12 years), smoking (ever or never), and parity (0 live births, 1 live birth, > 1 live birth).

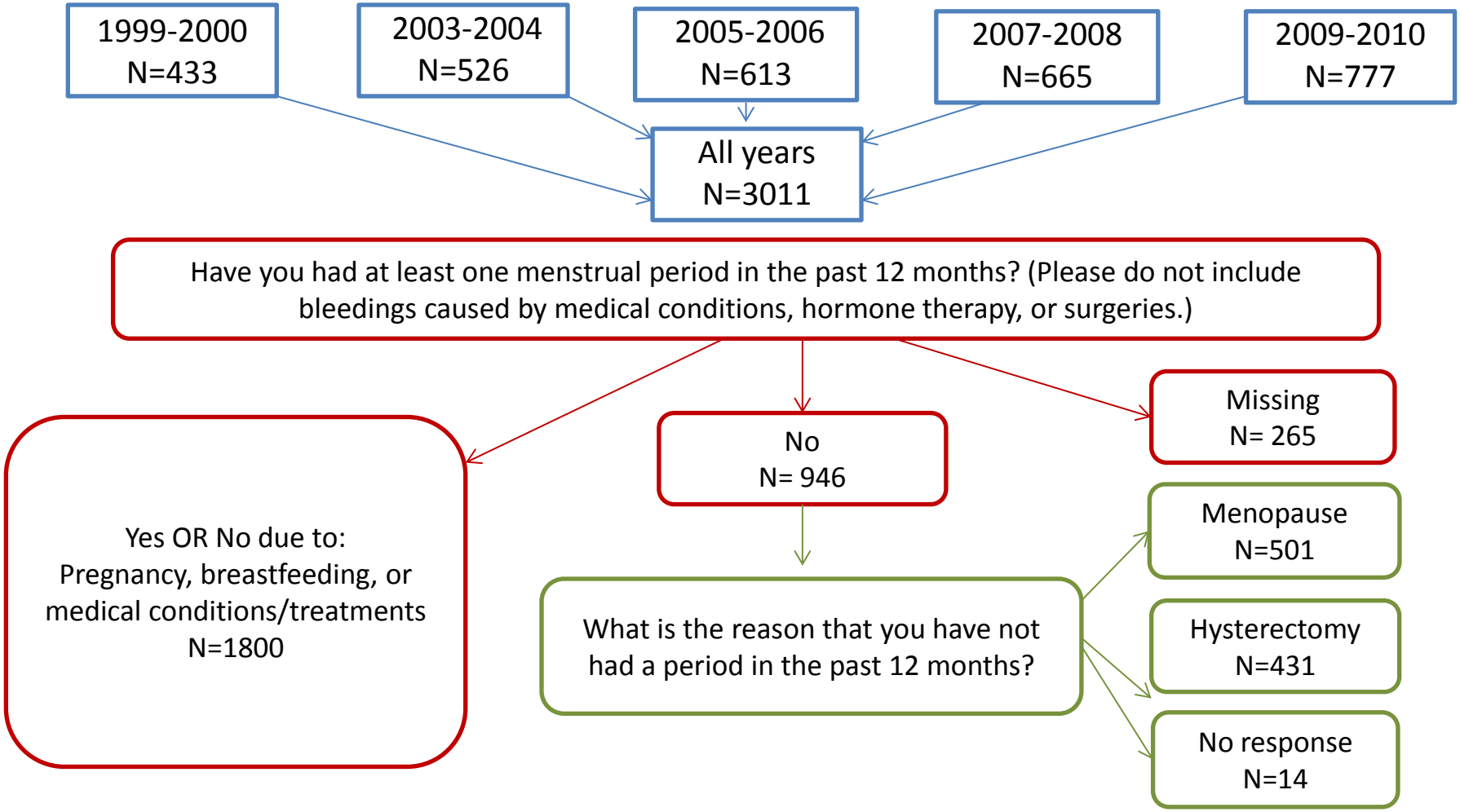

1999-2000
N=433
2003-2004
N=526
2005-2006
N=613
2007-2008
N=665
2009-2010
N=777
All years
N=3011
Have you had at least one menstrual period in the past 12 months? (Please do not include bleedings caused by medical conditions, hormone therapy, or surgeries.)
Yes OR No due to:
Pregnancy, breastfeeding, or medical conditions/treatments
N=1800
No
N= 946
Missing
N= 265
What is the reason that you have not had a period in the past 12 months?
Menopause
N=501
Hysterectomy
N=431
No response
N=14

Figure 1.

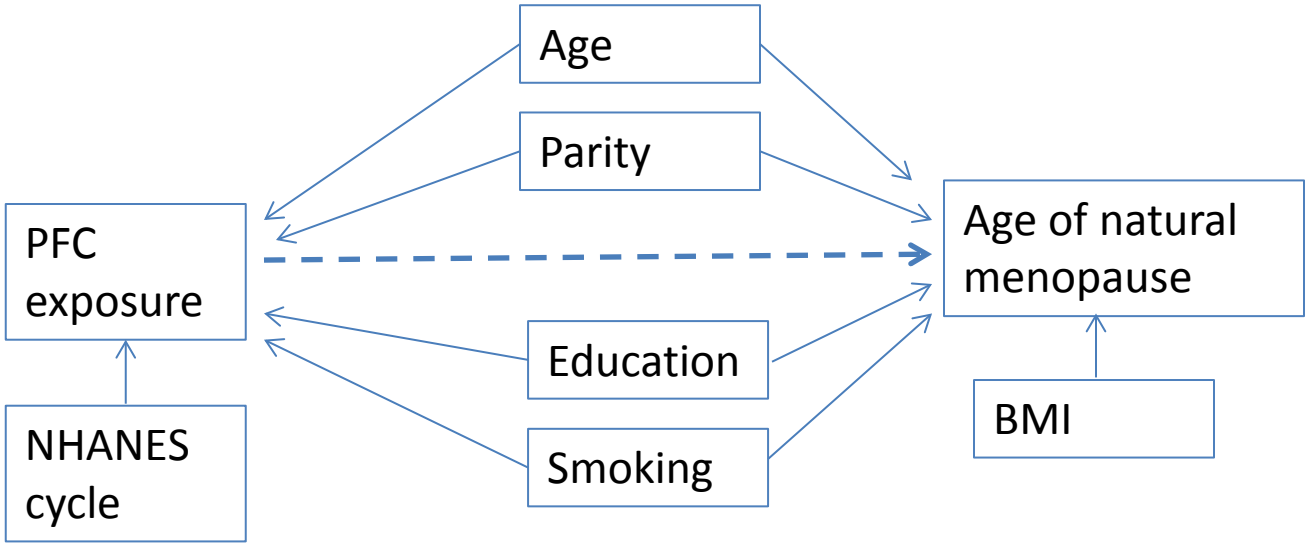
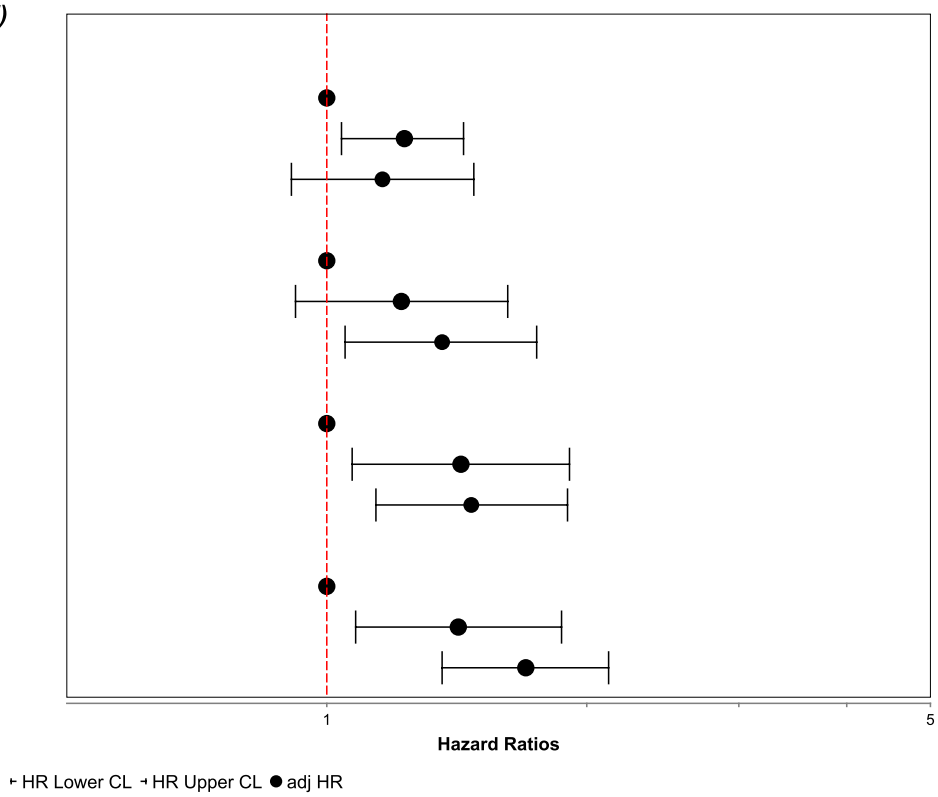

Age
Parity
PFC exposure
Age of natural menopause
Education
BMI
NHANES cycle
Smoking

Figure 2.

Exposure	*N*	*adj HR (95% CI)*
PFOS		
T1 (0.14 to 9 ng/mL)	634	1
T2 (>9 to 18.4 ng/mL)	756	1.23 (1.04, 1.44)
T3 (>18.4 ng/mL)	761	1.16 (0.91, 1.48)
PFOA		
T1 (0.07 to 2.5 ng/mL)	649	1
T2 (>2.5 to 4.4 ng/mL)	723	1.22 (0.92, 1.62)
T3 (>4.4 ng/mL)	779	1.36 (1.05, 1.75)
PFNA		
T1 (0.07 to 0.80 ng/mL)	668	1
T2 (>0.80 to 1.5 ng/mL)	712	1.43 (1.07, 1.91)
T3 (>1.5 ng/mL)	771	1.47 (1.14, 1.90)
PFHxS		
T1 (0.07 to 0.90 ng/mL)	621	1
T2 (>0.90 to 1.8 ng/mL)	715	1.42 (1.08, 1.87)
T3 (>1.8 ng/mL)	815	1.70 (1.36, 2.12)



Exposure	*N*	*adj HR (95% CI)*
PFOS		
T1 (0.14 to 9 ng/mL)	660	1
T2 (>9 to 18.4 ng/mL)	706	1.44 (1.12, 1.85)
T3 (>18.4 ng/mL)	733	2.56 (1.90, 3.43)
PFOA		
T1 (0.07 to 2.5 ng/mL)	658	1
T2 (>2.5 to 4.4 ng/mL)	696	1.83 (1.31, 2.56)
T3 (>4.4 ng/mL)	745	2.81 (2.12, 3.71)
PFNA		
T1 (0.07 to 0.80 ng/mL)	652	1
T2 (>0.80 to 1.5 ng/mL)	692	1.39 (1.08, 1.80)
T3 (>1.5 ng/mL)	755	1.78 (1.33, 2.37)
PFHxS		
T1 (0.07 to 0.90 ng/mL)	633	1
T2 (>0.90 to 1.8 ng/mL)	683	2.22 (1.66, 2.98)
T3 (>1.8 ng/mL)	783	3.50 (2.72, 4.50)